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Speciation analysis of arsenic in prenatal and children's dietary supplements using microwave-enhanced extraction and ion chromatography–inductively coupled plasma mass spectrometry

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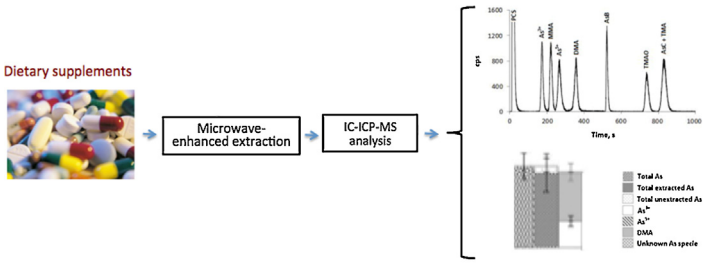
HIGHLIGHTS

- Microwave-enhanced extraction of arsenic from plant-based dietary supplements.
- Ion chromatography inductively plasma mass spectrometry method for arsenic.
- Optimal use of collision cell to eliminate molecular ions interfering with ^{75}As.
- Analysis of widely consumed prenatal and children's dietary supplements.
- Validation of analytical data using mass balance.

GRAPHICAL ABSTRACT



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ABSTRACT

A study was conducted to develop a microwave-enhanced extraction method for the determination of arsenic species in prenatal and children's dietary supplements prepared from plant materials. The method was optimized by evaluating the efficiency of various solutions previously used to extract arsenic from the types of plant materials used in the dietary supplement formulations. A multivitamin standard reference material (NIST SRM 3280) and a prenatal supplement sample were analyzed in the method optimization. The identified optimum conditions were 0.25 g of sample, 5 mL of 0.3 mol L^{-1} orthophosphoric acid (H_3PO_4) and microwave heating at 90 °C for 30 min. The extracted arsenic was speciated by cation exchange ion chromatography–inductively coupled plasma mass spectrometry (IC–ICP-MS). The method detection limit (MDL) for the arsenic species was in the range 2–8 ng g^{-1}. Ten widely consumed prenatal and children's dietary supplements were analyzed using the optimized protocol. The supplements were found to have total arsenic in the concentration range 59–531 ng g^{-1}. The extraction procedure recovered 61–92% of the arsenic from the supplements. All the supplementary products were found to contain arsenite (As^{3+}) and dimethylarsinic acid (DMA). Arsenate (As^{5+}) was found in two of the supplements, and an unknown specie of arsenic was detected in one product. The results of the analysis were validated using mass balance by comparing the sum of the extracted and non-extracted arsenic with the total concentration of the element in the corresponding samples.

1. Introduction

The human diet provides a diverse blend of nutrients needed for growth, maintenance and overall health. For some people, however,

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food alone may not supply adequate amounts of the required nutrients. Furthermore, nutritional needs change with age, pregnancy, specific metabolism and lactation, or due to acute and chronic diseases and other medical conditions. Pregnant women take dietary supplements that contain macro- and micronutrients to decrease the risk of several complications including congenital malformations, maternal anemia and preeclampsia, thereby ensure safe pregnancy and healthy babies [1]. Supplements are also given to children who are at risk of nutrient deficiency due to lack of appetite or highly selective diet. The demand for such supplementary products has increased significantly especially in the industrialized areas including the US, Canada and Europe [2].

Dietary supplements are prepared through laboratory synthesis or from natural products such as plants and fish oil that are characterized by high contents of vitamins, minerals and other essential nutrients [3]. The products are often considered to be exclusively beneficial to health and free from toxic side effects. However, studies found high levels of toxic and xenobiotic elements [4–10], pesticides [11] and bacteria [5] in some types of supplements. Exposure of a population, especially pregnant women and children, to such substances is a major concern. This concern would be even more troubling if the exposure occurs through dietary supplements; a source unexpected by the public.

Arsenic is among the elements of primary concern due to the toxicity of some of its species. Contamination of dietary supplements by arsenic can result mainly through the plants that are used as product ingredients. Arsenic is released into the environment through natural processes such as weathering of minerals, volcanic activity and soil erosion [12], as well as anthropogenic activities including mining and ore smelting, coal combustion, waste incineration, and use of pesticides [13]. Plants take up arsenic from soil and water, and accumulate it in their edible parts mainly as arsenate, which can cross the plasma membrane as a phosphate analogue [14]. In addition to the raw materials, the manufacturing steps which include extraction, formulation, etc. may also contribute to the contamination of the supplement products.

Several regulatory bodies have emphasized the need to focus on dietary arsenic exposure. The United Nations' Food and Agricultural Organization (FAO), and World Health Organization (WHO) set benchmark dose levels of 0.3–8 μg per kg of body weight per day for inorganic arsenic, i.e. arsenite (As^{3+}) and arsenate (As^{+5}), associated with risks for several diseases [15]. Arsenic is also one of the toxic substances listed by the US Environmental Protection Agency (EPA) [16] and in the State of California Proposition 65 [17] with a limit of 10 μg per day. The Chinese regulation has established a tolerance limit of 0.3 mg kg^{-1} for arsenic in dietary supplements [18]. Although the European Union has recently set maximum levels for several toxic elements in relation to contamination of food supplements, no value has been put for arsenic [19].

So far, a limited number of studies evaluated the level of arsenic in dietary supplements [5–7,9,10,20,21] mostly based on the determination of the total concentration of the element in the products [5–7,20,21]. Such studies, however, should be conducted based on the determination of the individual species of the element because the toxicity of arsenic depends on its chemical forms. For example, long-term exposure to inorganic arsenic is associated with a range of adverse effects on humans, including skin lesions, cancer, developmental toxicity, neurotoxicity, cardiovascular diseases, abnormal glucose metabolism and diabetes [22]. The methylated forms of arsenic such as monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) have been labeled as cancer promoters [23,24]. Arsenobetaine (AsB) and arsenocholine (AsC) are believed to be virtually nontoxic, however, this belief is still up for debate due to recent findings [25]. To the authors' best knowledge, no work has been reported yet on the comprehensive speciation analysis of arsenic in dietary supplements, except for two studies which aimed only at the inorganic species of the element with [9] or without [10] their differential determination.

Table 1
Optimum conditions of the chromatographic method.

Parameter	Optimum condition
Column	PRP-X200 (cation exchange), 250 mm long, 4.1 mm i.d., 10 μm particle size, working pH 1–9
Mobile phases	(A) 1 mmol L^{-1} HNO_3 and 1% methanol in water, pH 2.5, and (B) 2 mmol L^{-1} HNO_3, 20 mmol L^{-1} ammonium nitrate and 1% methanol in water, pH 2.5
Elution mode	Gradient: 0.0–3.0 min (95% A and 5% B), 3.1–16.0 min (5% A and 95% B), 16.1–18.0 min (95% A and 5% B)
Flow rate	0.9 mL min^{-1}
Column temperature	Ambient
Injection volume (sample and post-column standard)	100 μL

The study presented in this paper aimed to develop an extraction method for the comprehensive speciation analysis of arsenic in prenatal and children's dietary supplements prepared from plant materials such as fruits, vegetables, grains and herbs. Numerous methods have been reported for extracting arsenic species from environmental, nutritional, botanical, biological and other samples [26,27], but none exists for samples of the type considered in the present study. Hence, the novelty of this study resides in the development of the extraction protocol and its application in supplement materials produced for vulnerable population subgroups, i.e. children and pregnant women. The method was developed based on a microwave-enhanced protocol. Several solutions previously used to extract arsenic species from the types of plant materials used in the dietary supplement formulations were evaluated by analyzing a multivitamin standard reference material and a prenatal supplement sample. The extracted arsenic was speciated by cation exchange ion chromatography–inductively coupled plasma mass spectrometry (IC–ICP-MS). Several widely consumed prenatal and children's supplements were analyzed using the proposed method. The analytical results were validated using mass balance by comparing the sum of the concentrations of the extracted and non-extracted arsenic species with the total arsenic found in the corresponding samples.

2. Experimental

2.1. Instrumentation and software

Extraction and sample decomposition were performed using an Ethos 1 laboratory microwave system (Milestone). The instrument was equipped with temperature and pressure feedback control and magnetic stirring capability. The device accurately senses within ±2.0 °C of the set temperature, and automatically adjusts the microwave field output power.

A SAVANT SPD1010 SpeedVac concentrator (Thermo Scientific) was used solvent for solvent evaporation.

Electrochemical potential (E_h) measurement was made with a Keithley 169 multimeter using a Pt-electrode (Metrohm AG) against a saturated Calomel electrode (Accument).

The ion chromatographic system was Metrohm 850 Professional IC (Metrohm). The system's hardware was made from PEEK, and it consisted of an auto-sampler (858 professional sample processor), a six-port sample injector, two pumps, a column thermostat, an eluent degasser, and an automated post-column injection unit (800 Dosino). A PRP X-200 cation exchange column (Hamilton) was used. Table 1 provides the optimum chromatographic conditions.

An Agilent 7700x ICP-MS (Agilent Technologies) equipped with a micro-mist nebulizer, a quartz spray chamber, an octopole reaction system (ORS^3), and a quadrupole mass analyzer was used. The argon and helium were of ultra high purity grade (99.999%, Airgas). The operating conditions of the instrument were RF power (1550 W), RF matching (1.8 V), sampling depth (8 mm) and flow of plasma, carrier and makeup gas (15, 0.95 and 0.15 L min^{-1}, respectively). The instrument was tuned on the day of every analysis using an Agilent tuning solution that contained 1 μg L^{-1} Li, Co, Y, Ce, and Tl in 2% HNO_3. Sensitivity, calculated as million counts-per-second (Mcps) per mg L^{-1}, of 40 (^{7}Li), 130 (^{89}Y) and 65 (^{205}Ti) or higher was achieved. The maximum oxide (CeO/Ce) and doubly charged (Ce^{2+}/Ce) ratios were 1.16% and 0.76%, respectively.

For direct analysis by ICP-MS, samples were introduced using an auto-sampler (ASX-500 Series, Agilent Technologies), which was kept in an anti-contamination enclosure (ENC500, CETAC Technologies). For the IC–ICP-MS configuration, the IC column outlet was connected to the ICP-MS nebulizer inlet using a switching valve. The ICP-MS sample line and the IC injection unit were rinsed prior to each sample analysis in a three-step procedure using 1% HC1, 1% HNO_3, and ultrapure water.

Agilent MassHunter software (version G7201A A.01.01, Agilent Technologies) was used for ICP-MS data acquisition, chromatographic peak integration and analyte quantification.

2.2. Chemicals and working solutions

All the reagents were of analytical or ultra-pure grade. The reagents and suppliers were HNO_3 (69%), HCl (32–35%), orthophosphoric acid (H_3PO_4, 85%), methanol (99.9%), acetonitrile (99.9%) and ammonium oxalate from Fisher Scientific; ammonium nitrate and Streptomyces griseus' protease XIV from Sigma–Aldrich; H_2O_2 (30%, EMD), ammonium hydroxide solution (25–28%, BDH), trifluoroacetic acid (Alfa Aesar), tetramethyl ammonium hydroxide solution (25%, Acros Organics), α-amylase from Bacillus subtilis (Fluka), and ultrapure water (18.2 MΩ cm, Branstead NANOpure).

Stock solutions (1000 mg L^{-1} as arsenic) of As^{3+} and As^{5+} from SPEX CentriPrep were used. Standard solutions (1000 mg L^{-1} as arsenic) of arsenobetaine (AsB), arsenocholine (AsC), dimethylarsinic acid (DMA), monomethylarsinic acid (MMA), tetramethylarsonium (TMA) and trimethylarsine oxide (TMAO) were prepared by dissolving appropriate amounts of arsenobetaine (Argus Chemicals), arsenocholine (Argus Chemicals), sodium cacodylate trihydrate (Sigma–Aldrich), disodium methyl arsonate hexahydrate (Chem Service), tetramethylarsonium iodide and trimethylarsine oxide (Argus Chemicals), respectively, in water. All stock solutions were prepared in Teflon bottles. All chemicals and standards were stored in a clean cold-room at 4 °C, away from ultraviolet lamps and sunlight.

2.3. Dietary supplement samples and standard reference material

Seven prenatal (P1–P7) and three children's dietary supplements (C1–C3) were purchased from local retail outlets in Pittsburgh, Pennsylvania, USA. The major ingredients of all the products were plant materials. The supplements were available in tablet, capsule or liquid forms. A multivitamin standard reference material (SRM 3280) from National Institute of Standards and Technology (NIST) was used for method optimization and validation. Table 2 shows the list of the supplements and their major plant ingredients. All procedures pertaining to the homogenization, preparation and analysis of the samples were carried out in a clean room equipped with a class-100 high efficiency particulate air filter hood.

Table 2
List of prenatal and children's dietary supplements and a standard reference material analyzed in the present study.

Supplement type	Company	Sample number	Plant materials used as major ingredients[a,b]
Prenatal	A	P1	Tomato, grape seed, pomegranate, orange, ginger, spinach, blueberry, cranberry, peppermint, broccoli, soybean
Prenatal	A	P2	Vegetables (names not listed)
Prenatal	A	P3	Vegetables (names not listed)
Prenatal	B	P4	Strawberry, cherry, blackberry, blueberry, raspberry, beet, carrot, spinach, broccoli, tomato, kale, red cabbage, parsley, brussels sprout, green bell pepper, cucumber, celery, garlic, ginger, green onion, cauliflower, asparagus
Prenatal	C	P5	Brown rice, oat, blueberry, prunes, blackberry, flame raisin, raspberry, dandelion, rose hips, lavender, lemon balm, peppermint, cloves, sunflower
Prenatal	D	P6	Fruits and vegetables (names not listed)
Prenatal	E	P7	Grape, vegetables (names not listed)
Children's	A	C1	Bean, fruits (names not listed)
Children's	F	C2	Cherry and other naturals (names not listed)
Children's	G	C3	Brown rice, mixed berry, raspberry, vanilla, cherry, ultra green foods concentrate, peppermint, spinach, chamomile, licorice, nettles, anise, millefolium, plantago, equisetum, green tea, alfalfa
Standard reference material	NIST	SRM 3280	–

[a] Supplements contained extracts, powders, juices and/or oils generated from the leaves, flowers, roots, fruits, skins and/or sprouts of the plants.
[b] Other inactive ingredients: cellulose (in all supplements except P5, C1 and C2), titanium dioxide (in P1–P3, P6 and P7), silica (in P5–P7 and C2), and vegetable acetoglycerides (in P1–P3).

2.4. Sample homogenization

The supplement products and the SRM were homogenized for sample preparation (digestion and extraction) according to the procedure described in the certificate of analysis for SRM 3280. For capsules, the contents of fifteen samples were emptied and homogenized with mortar and pestle. For tablets, fifteen samples were directly pulverized with mortar and pestle. The ground samples were transferred into polyethylene (PE) tubes and stored at room temperature.

2.5. Sample decomposition for total arsenic determination

The supplement samples were digested according to EPA Method 3052 [28] to determine their total arsenic content. Three replicate digests were prepared per sample as follows. 0.25 g of a supplement sample was weighed out into three microwave vessels, and 8.0 mL of conc. HNO_3 and 2.0 mL of H_2O_2 were added into each vessel. The mixtures were swirled to ensure wetting and mixing,

and the vessels were sealed after putting magnetic stirrers. The mixtures were irradiated in the microwave system at 180 °C for 10 min with a ramp time of 5 min. The sample digests were cooled to room temperature, diluted with ultrapure water, and centrifuged at 3600 rpm for 20 min. The supernatants were decanted into clean PE tubes and stored in a clean cold-room at 4 °C. Three method blanks were also prepared.

2.6. Method optimization and extraction of arsenic species

Optimization of the extraction method was carried out by analyzing SRM 3280 and sample P3 (Table 2) as follows. For each sample, 0.25 g was weighed into three microwave vessels followed by addition of 5.0 mL of the extraction solution (see Table 4), and irradiation in a microwave. Enzymatic extractions with α-amylase and protease XIV were conducted at 37 °C for 40 min, and extraction with methanol–water mixture at 50 °C for 30 min. The rest of the extractions were conducted at 90 °C for 30 min. The microwave programs of all extractions had 5 min ramp time. Extracts were cooled, centrifuged (3600 rpm, 20 min), and the supernatants were decanted, filtered and stored in a clean cold room at 4 °C. Three method blanks were also prepared for each extraction protocol.

The optimum condition (see Section 3.3.9) was used to extract the arsenic species from all the dietary supplement samples listed in Table 2. Four extracts (three unspiked and one spiked) were generated from each supplement as follows. 0.25 g of a sample was weighed out into four microwave vessels, and 50 μL standard (10 mg L^{-1}) of each of the arsenic specie was spiked into one of the vessels. Then, 5.0 mL of 0.3 M H_3PO_4 was added into each of the vessels, magnetic stirrers were put and the mixtures were irradiated in a microwave at 90 °C for 30 min. Extracts were cooled, centrifuged (3600 rpm, 20 min), and the supernatants were decanted, filtered and stored at 4 °C. Three method blanks were also prepared.

The residues left from extraction of the unspiked samples were transferred into microwave vessels and digested according to EPA Method 3052 [28] (as described in Section 2.5) to determine the non-extracted arsenic in the corresponding samples as total non-extracted arsenic.

2.7. Analysis of sample digests and extracts

Digests generated from the supplement samples and the extraction residues, and extracts of the unspiked samples were analyzed by ICP-MS after appropriate dilution to determine the concentrations of total, non-extracted and extracted arsenic, respectively. The extracts were also analyzed by IC–ICP-MS to speciate the extracted arsenic. Analytes were quantified by external calibration using matrix-matched standards prepared in the corresponding method blanks. For the IC–ICP-MS analyses, calibration curves were constructed on the basis of peak area. Calibrations were verified by analyzing a test standard solution after every five samples in the analysis sequence. In addition, a post-column standard (PCS) solution of 10.0 ng g^{-1} As^{5+} was used to monitor the stability of the IC–ICP-MS system.

2.8. Method detection limit (MDL)

The MDL was determined according to the procedure given in the Code of Federal Regulations [29]. An aqueous solution containg 2.0 mg L^{-1} of each of the arsenic species was prepared, and 25.0 μL of the solution was transferred into seven microwave vessels. The samples were irradiated in a microwave (90 °C, 30 min) after adding 5.0 mL of 0.3 mol L^{-1} H_3PO_4. The extracts were analyzed by IC–ICP-MS after appropriate dilution.

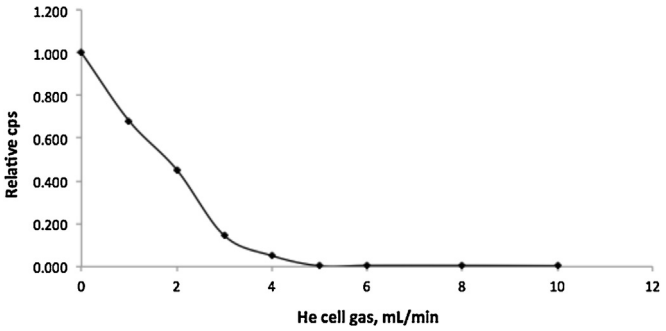

Fig. 1. Relative ion counts (cps_R) at m/z 75 for analysis of 2% (v/v) HCl solution by ICP-MS at different He gas flow rates.

3. Results and discussion

3.1. Removal of molecular ions interfering with ^{75}As

A number of molecular ions interfere with the detection of ^{75}As by ICP-MS [30,31]. The ORS^3 in Agilent 7700 ICP-MS eliminates such interferences by using an inert collision cell gas (He) and kinetic energy discrimination [31]. Most studies use the ORS^3 without thoroughly optimizing the gas flow. A randomly selected gas flow rate does not guarantee effective elimination of interferences, and can lead to erroneous results. In the present study, a condition was established for effective interference removal by analyzing a 2% (v/v) HCl solution at different flow rates of He (other instrument settings were kept as described in Section 2.1). The expected predominant interfering ions were $^{40}Ar^{35}Cl^+$, $^{38}Ar^{37}Cl^+$, $^{36}Ar^{38}Ar^1H^+$ and $^{37}Cl_2H^+$ [30,31]. A relative cps function (cps_R) was defined as $cps_R = cps_{He}/cps_0$; where; cps_{He} and cps_0 were ion counts at m/z 75 in He and no gas modes, respectively. Fig. 1 shows the cps_R values at m/z 75 as a function of the He flow rate. The cps_R value was lowered to zero at a He flow rate of 5.0 mL min^{-1} and higher. A flow rate of 5.0 mL min^{-1} was used throughout this study.

3.2. IC–ICP-MS method for the determination of arsenic species

Several chromatographic methods in combination with ICP-MS are available for the determination of arsenic species; anion exchange IC with carbonate- or phosphate-containing mobile phases are predominant [32,33]. These eluents, however, are not friendly for use in ICP-MS because carbonate leaves carbon deposits on the sampling and skimmer cones, [34] and phosphate causes clogging and rapid erosion of the cones [35]. A few studies demonstrated that ammonium nitrate is a better choice for use in ICP-MS because it does not cause clogging and interference problems [36–38]. In addition, it has minimal effect on the ionization characteristics of the plasma as the salt does not contain alkali or alkaline earth metals.

Ponthieu et al. [38] proposed a cation exchange IC–ICP-MS method for the simultaneous determination of eight arsenic species using mobile phases prepared from ammonium nitrate. The method separated As^{3+}, MMA, As^{5+}, DMA, AsB, and TMAO with partial overlap of the first two species. AsC and TMAO were separated from the other species, but resolution was not achieved between them. In the present study, this method was used after improving its performance with some modifications. Baseline separation was achieved between As^{3+} and MMA by decreasing the ionic strength and flow rate of the mobile phase. Also, the signal intensity of arsenic was enhanced by adding methanol into the eluents (1% v/v) as described elsewhere [36,37]. Table 1 lists the conditions of the modified method, and Fig. 2 shows a chromatogram for the separation of the arsenic species from a standard solution prepared in the

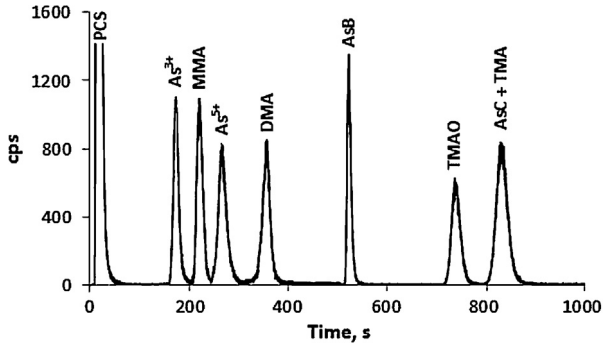

Fig. 2. Chromatogram showing the separation of arsenic species in a matrix-matched standard solution containing 2.0 ng g^{-1} arsenic per specie. See Table 1 for the chromatographic conditions. PCS: post-column standard.

method blank (Section 2.6, paragraph 2). The separation and order of elution of the species are governed by their relative charge and chemical composition. As can be deduced from the pK_a values and the chemical formulae of the species given in Table 3, As^{3+} and MMA are neutral at the eluent pH (2.5). Thus, the elution of these species is a function of their hydrophobic interaction with the column. AsC, TMAO and TMA, which exist as cations, stay in the column for longer times due to their interaction with the ion exchange site. DMA and AsB are zwitterions at pH of the chromatographic condition; hence, they leave the column earlier than the cations.

To monitor any shift in the retention times of the analytes due to high matrix effects, the supplement extracts were analyzed along with extracts generated from the corresponding spiked samples (see Section 2.6, paragraph 2).

3.3. Method optimization for the extraction of arsenic from dietary supplements

Several solutions were evaluated to develop the extraction method in a microwave-enhanced protocol. The solutions were water, methanol–water mixture, and aqueous solutions of α-amylase, α-amylase–protease mixture, HCl–H_2O_2 mixture, tetramethylammonium hydroxide, trifluoroacetic acid, ammonium oxalate and H_3PO_4. All solutions were previously applied to extract arsenic from some of the plants used in the formulations of the supplements (Table 2). Extraction using methanol–water mixture was conducted for 30 min keeping the temperature below the boiling point of methanol, at 50 °C. Since enzymatic extractions require moderate temperatures, extractions with α-amylase and protease XIV were conducted at 37 °C for 40 min as described elsewhere [40]. The rest extractions were carried out for 30 min keeping the temperature below the boiling point of water, at 90 °C. Preliminary experiments in this study and a previous report [41] showed that the target arsenic species are stable at 90 °C in water.

Since there is no standard reference material for arsenic species in dietary supplements, the extraction method was optimized by evaluating the recovery of total arsenic from SRM 3280 and a prenatal supplement (sample P3, Table 2). The total arsenic concentrations in the two samples were measured to be 128 ± 16 and 343 ± 29 ng g^{-1}, respectively. The performances of the extraction conditions are discussed in the following subsections, and the results are summarized in Table 4.

Table 3
pK_a values of arsenic species [39].

Specie	Chemical formula	pK_a
Arsenite (Arsenous acid), As^{3+}	$AsO(OH)$	9.29
Arsenate (Arsenic acid), As^{5+}	$AsO(OH)_3$	2.24, 6.96, 11.5
Dimethylarsinic acid, DMA	$(CH_3)_2As^+(OH)_2$	1.78, 6.14
Monomethylarsonic acid, MMA	$CH_3AsO(OH)_2$	3.6, 8.2
Arsenobetaine, AsB	$(CH_3)_3As^+CH_2COOH$	2.18
Arsenocholine, AsC	$(CH_3)_3As^+(CH_2)_2OH$	–
Trimethylarsine oxide, TMAO	$(CH_3)_3As^+OH$	3.6
Tetramethylarsonium ion, TMA	$(CH_3)_4As^+$	–

Table 4
Percentage of total arsenic extracted from SRM 3280 and a prenatal supplement (sample P3) using the extraction conditions evaluated in this study (n = 3, 95% CL).

Extraction solution	Extraction recovery (%)[a]	
	SRM 3280	Sample P3
Water	2.9 ± 1.8	23.3 ± 7.6
25% Methanol in water	1.8 ± 0.9	8.3 ± 2.3
α-Amylase	1.1 ± 0.2	23.9 ± 9.7
α-Amylase and protease XIV	0.9 ± 0.1	16.1 ± 2.8
0.06 M HCl and 3% H_2O_2	45.2 ± 6.7	15.0 ± 3.1
5% Tetramethylammonium hydroxide	34.1 ± 5.6	25.9 ± 17.2
0.2 M Trifluoroacetic acid	58.9 ± 7.6	11.1 ± 3.4
0.2 M Ammonium oxalate	22.4 ± 5.4	48.5 ± 9.6
0.3 M H_3PO_4	95.5 ± 8.6	63.5 ± 7.2

[a] Extraction recovery (%) = (extracted arsenic/total arsenic) × 100.

3.3.1. Water

Water has been demonstrated to be a suitable solvent to extract the polar species of arsenic from various types of samples including plants [26,27]. Results in the present study, however, showed that only 2.9% and close to one-fourth of the total arsenic were solubilized in water from the SRM and sample P3, respectively.

3.3.2. Methanol–water mixture

Several studies used mixtures of methanol and water at varying proportions (10–50% methanol) to extract arsenic from vegetables [42], herbs [43], rice [44] and other grains [45,46]. Methanol is added to water with the intention of increasing the solubility of the non-polar arsenic species. In most cases, multiple extractions are employed to improve the extraction efficiency by pipetting off the supernatant and re-extracting the wet pellet with a fresh solvent. It was, however, found that the apparent 'additional' arsenic in the subsequent extractions comes mainly from residual dissolved arsenic carried over from the first extraction [26,27].

A single step extraction using 25% methanol (in water) was carried out in the present study. After microwave extraction, the methanol was removed from the extract using a SpeedVac concentrator and the aqueous portion of the extract, which contains the solubilized species, was analyzed. As can be seen from Table 4, the methanol–water mixture extracted lower amount of arsenic from both samples compared to water. The decrease in the extraction recovery is probably because the samples had very low concentration of non-polar arsenicals, and also the methanol decreased the solubility of the polar arsenic species.

3.3.3. Enzymatic solutions

Enzymatic solutions of α-amylase with or without protease have been used to extract arsenic from apple [47,48] and rice [40,49,50]. α-Amylase hydrolyses the α-1,4-linkage of starch thereby liberating proteins and facilitating the solubilization of protein-bound arsenic, and protease eases the release of arsenic by hydrolyzing the proteins to peptides and amino acids [49].

Extractions were made using α-amylase solutions with and without protease; 25 mg of α-amylase, 50 mg of protease XIV and 5 mL of water were used with a 0.25 g sample. The mixtures were irradiated in a microwave as described elsewhere [40]. Table 4 shows that the amount of arsenic extracted from SRM 3280 and sample P3 by the enzymes were less than or almost equal to the water-soluble fractions of the element. Some studies reported that extraction using 25% methanol or acetonitrile following the

enzymatic extraction provides better recovery [47,48,51]. The strategy was tested in this study using 25% acetonitrile in water but it did not improve the recovery.

3.3.4. HCl–H_2O_2 mixture

Hedegaard et al. [10] used an aqueous solution containing HCl (0.06 mol L^{-1}) and H_2O_2 (3%) to determine inorganic arsenic in dietary supplements prepared from herbs and other botanicals. The study reported that apart from oxidizing As^{3+} to As^{5+}, the extraction condition did not impose any effect on the organoarsenic compounds. The condition was used in the present study, but only 45% and 15% of the total arsenic were extracted from SRM 3280 and sample P3, respectively.

3.3.5. Tetramethylammonium hydroxide

Tetramethylammonium hydroxide is a basic extractant that has been frequently used to solubilize arsenic species from biological tissues [51]. A few studies also used this reagent for extracting arsenic from some plant materials [52]. In the present study, aqueous solutions containing 1–5% of the reagent were used but not more than 35% of the total arsenic was recovered from the two test samples (Table 4).

3.3.6. Trifluoroacetic acid

A few studies reported the use of trifluoroacetic acid to extract arsenic from rice [50,53]. In the present study, a series of aqueous solutions containing 0.02–0.2 mol L^{-1} of the acid were evaluated. The maximum recoveries obtained from SRM 3280 and sample P3 were 60% and 10%, respectively, using 0.2 mol L^{-1} trifluoroacetic acid.

3.3.7. Ammonium oxalate

Ammonium oxalate has been demonstrated to be effective in solubilizing arsenic from samples having crystallized iron oxide; a strong arsenic adsorbent [54]. Since all the dietary supplements were found to have very high concentration of iron (0.5–8.4 mg g^{-1}), the use of solutions containing this reagent was assumed to be helpful in achieving better extraction recovery. Accordingly, a series of solutions containing 0.02–0.2 mol L^{-1} of the reagent were evaluated. The 0.2 mol L^{-1} solution provided better extraction from both samples, but the recoveries were only 22% (SRM 3280) and 49% (sample P3).

3.3.8. Orthophosphoric acid

Solutions of H_3PO_4 are reported to be efficient in extracting arsenic from several plant materials including vegetables, rice and beans [55]. In the present study, 0.1–0.5 mol L^{-1} H_3PO_4 solutions were used to extract arsenic from the two test samples. While all the H_3PO_4 solutions extracted 84%–96% of the total arsenic from SRM 3280, only the 0.3 mol L^{-1} solution provided better recovery (63%) from sample 3.

Among the solutions tested in the method optimization, H_3PO_4 provided high extraction recoveries from both test samples. The better performance of the acid is possibly because it can break As—S bonds [55]. Furthermore, phosphate can desorb arsenate from the matrix because of the similarities in the physicochemical properties of the two species.

3.3.9. Optimum extraction condition

Based on the results found in the above sub-sections, a solution of 0.3 mol L^{-1} H_3PO_4 was used as an optimum extractant to study the speciation of arsenic in the dietary supplements. Details of the extraction procedure are described in Section 2.6, paragraph 2.

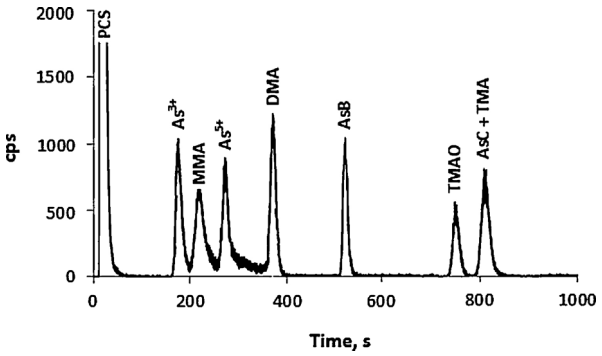

Fig. 3. Chromatogram for an extract generated from a prenatal supplement (sample P5) spiked with the target arsenic species. See Table 1 for the chromatographic conditions. PCS: post-column standard.

3.4. Effect of the optimum extraction condition on the stability of arsenic species

The effect of the optimum extraction condition on the stability of the arsenic species was evaluated using a prenatal supplement (sample P5) spiked with the arsenic species (see Section 2.6 for the extraction procedure). Fig. 3 shows the chromatogram for the extract generated from the spiked sample. All the arsenic species were identified with spike recoveries of 83–109%. The observed stability of the analytes was apparent from the measured pH (5.2) and Eh (+0.004) of the extract, and the redox distribution of the species [56]. The increase in the pH of the extract (compared to that of the extractant, pH 1.4) is possibly due to the solubilization of reducing substances (such as antioxidants) from the supplement. Antioxidants are often added into such products with the aim of protecting the human body from harmful free radicals. The results of this experiment show that H_3PO_4 is a good choice due to its less significant effect on the species. Moreover, exposure assessment using this extractant can provide valuable insight in relation to human health, because phosphate is a natural component of human body found in blood and other organs playing roles in the production and storage of energy, skeletal mineralization and removal of waste from kidney.

3.5. Method detection limit

The MDL was determined according to the procedure given in the US Code of Federal Regulations [29]. In this document, MDL is defined as the minimum concentration of a substance that can be measured and reported with 99% CL that the analyte concentration is greater than zero, and is determined from analysis of a sample in a given matrix containing the analyte. Seven extracts were prepared along with a reagent blank as described in Section 2.8. The extracts were analyzed by IC–ICP-MS and the MDL for each of the analytes was calculated as 3.143 times the standard deviation of the seven concentration readings. With 1% methanol in the mobile phase and a 100 μL sample injection volume, the analyte species down to 2–8 ng g^{-1} (as arsenic) could be detected using this method.

3.6. Analysis of dietary supplement samples

Total arsenic, individual arsenic species and the non-extracted fraction of arsenic were determined in seven prenatal and three children's dietary supplements (Table 2) as discussed in the following sub-sections.

3.6.1. Determination of total arsenic

The total arsenic concentration in the supplements was determined by ICP–MS after decomposing the samples using a

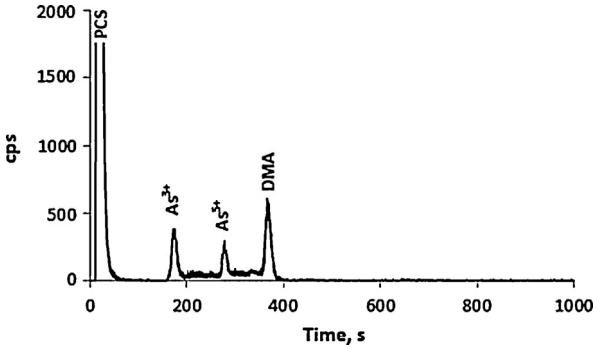

Fig. 4. Chromatogram for an extract generated from a prenatal supplement (sample P5). See Table 1 for the chromatographic conditions. PCS: post-column standard.

microwave (Section 2.5). SRM 3280 was analyzed to validate the analytical procedure. An arsenic concentration of $128 \pm 16\ ng\ g^{-1}$ was found in the SRM, which is in good agreement with the certified value ($132 \pm 44\ ng\ g^{-1}$) at 95% CL. The total arsenic found in the supplement samples ranged from 59 to 531 $ng\ g^{-1}$ (Table 5, column 2).

3.6.2. Determination of arsenic species

The arsenic in the supplement samples was extracted using the microwave-enhanced extraction method optimized in this study (Section 2.6). The extracts were analyzed by ICP-MS and IC–ICP-MS to determine the total extracted arsenic and the individual arsenic species, respectively. Fig. 4 shows a chromatogram for the analysis of an extract generated from a prenatal supplement (sample P5).

Comparison of the total arsenic found in the supplements with the corresponding total extracted arsenic indicates that the extraction protocol provided a recovery of 61–92% (Table 5, column 4). The non-quantitative extraction may be due to the binding of some forms of arsenic to insoluble incipiencies such as cellulose, titanium dioxide and silica [57] (see Table 2 for the list of inactive ingredients present in the dietary supplements). As can be seen from Table 5, the extraction recoveries found for most of the samples were greater than 80%. These recovery values should be acceptable to determine the highly mobile and potentially toxic species of arsenic in the samples. The lower recoveries may be improved by modifying some of the extraction parameters by adding substances that can desorb the strongly bound species, increasing the heating time, and/or increasing the extractant volume. However, as shown in the method optimization (Section 3.3.8), changing the concentration of the extractant (H_3PO_4) may not be a viable option.

The concentrations of the individual arsenic species determined in the extracts are also presented in Table 5. It was found that all the supplements contained As^{3+} ($20.0–127\ ng\ g^{-1}$) and DMA ($16.5–153\ ng\ g^{-1}$). As^{5+} was found in two of the samples at concentrations of $21.5\ ng\ g^{-1}$ (sample P5) and $67.0\ ng\ g^{-1}$ (sample C2) while an unknown arsenic species was detected in one sample (sample C2). Quantification of the unknown species based on the calibration of DMA gave a concentration of $114\ ng\ g^{-1}$. The arsenic species found in the supplements showed different levels and distribution; DMA predominates in most of the samples and As^{3+} in some.

3.6.3. Determination of non-extracted arsenic

The arsenic species that were not extracted from the samples were determined by decomposing the residues left from extraction, as described in Section 2.6. The digests were analyzed by ICP–MS to quantify the non-extracted species collectively as 'total non-extracted arsenic'. The results are presented in Table 5, column 5.

3.6.4. Mass balance

Two mass balance comparisons were made to validate the results of this study, Fig. 5. The first comparison was between the total concentration of arsenic with the sum of the concentrations of total extracted and non-extracted arsenic. The figure shows that good agreement was found between the two sets of measurements for all the samples. The second comparison was between the total extracted arsenic and the sum of the concentrations of the individual arsenic species. It can be seen from Fig. 5 that the sum of the individual species represented 66–106% of the total extracted arsenic. The non-quantitative chromatographic recovery for some of the samples may be due to the strong retention of some extracted arsenic species in the column. It was noticed during this study that some species of arsenic such as roxarsone could not be eluted from the separation column using the optimized composition.

3.7. Assessment of exposure to arsenic from the dietary supplements

The maximum exposure of pregnant women and children to arsenic through the consumption of the dietary supplements was calculated by multiplying the mass of the daily serving size of the supplement with the concentration of arsenic found in product,

Table 5
Concentrations ($ng\ g^{-1}$) of total, extracted and unextracted arsenic in the dietary supplements, and extraction recovery (%) values ($n = 3$, 95% CL).

Sample number	Total arsenic	Total extracted arsenic[a]	Extraction recovery (%)[b]	Total non-extracted arsenic[c]	Extracted arsenic species[d]			
					As^{3+}	As^{5+}	DMA	Unknown
P1	296 ± 17	239 ± 34	80.7 ± 16.1	40.3 ± 11.1	57.5 ± 8.8	ND	153 ± 16	ND
P2	226 ± 26	185 ± 9	81.9 ± 13.4	36.3 ± 11.6	20.3 ± 3.6	ND	120 ± 5	ND
P3	343 ± 29	212 ± 42	61.8 ± 17.5	136 ± 27	35.6 ± 9.4	ND	121 ± 31	ND
P4	531 ± 34	369 ± 22	69.5 ± 8.6	176 ± 16	127 ± 20	ND	117 ± 21	ND
P5	248 ± 20	219 ± 25	88.3 ± 17.2	21.1 ± 7.6	57.1 ± 5.7	21.5 ± 2.5	106 ± 8	ND
P6	197 ± 14	140 ± 4	71.1 ± 7.1	52.9 ± 10.8	27.1 ± 2.2	ND	121 ± 7	ND
P7	191 ± 24	116 ± 10	60.7 ± 12.9	72.1 ± 22.6	20.0 ± 4.5	ND	77.4 ± 8.5	ND
C1	144 ± 24	131 ± 12	91.0 ± 23.5	13.6 ± 5.5	46.1 ± 9.5	ND	86.7 ± 15.6	ND
C2	510 ± 35	438 ± 29	85.9 ± 11.6	61.5 ± 10.0	35.2 ± 1.5	67.0 ± 8.3	124 ± 6	114 ± 26
C3	59.2 ± 4.4	54.0 ± 6.1	91.2 ± 17.1	9.8 ± 3.3	25.0 ± 2.1	ND	16.5 ± 2.3	ND

[a] Total arsenic concentration in the extracts determined by ICP-MS.
[b] Extraction recovery (%) = (total extracted arsenic/total arsenic) × 100.
[c] Total non-extracted arsenic determined by ICP-MS after digesting the extraction residues.
[d] Individual arsenic species determined by microwave-enhanced extraction using H_3PO_4 and IC–ICP-MS analysis.
ND: not detected.

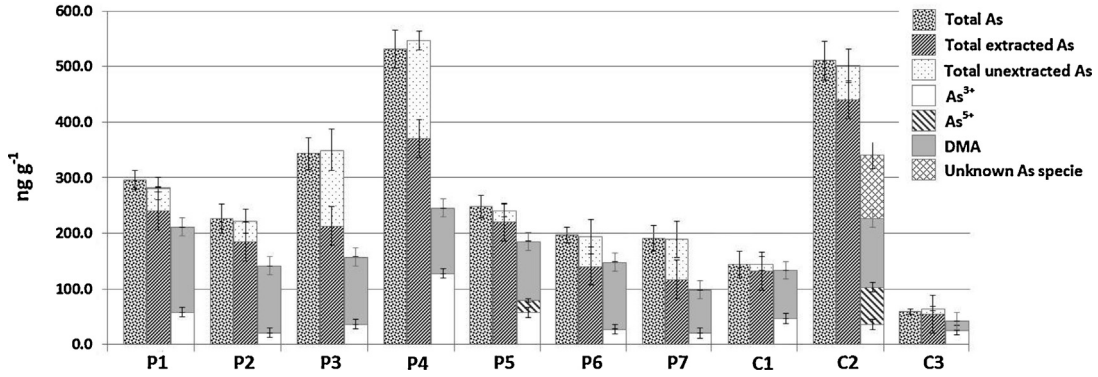

Fig. 5. Mass balance comparison of the measured concentrations of total, extracted and non-extracted arsenic in the prenatal and children's dietary supplements ($n = 3$, 95% CL).

Table 6
Exposure of pregnant women and children to total, extractable and individual arsenic species through the dietary supplements ($n = 3$, 95% CL).

Sample number	Dosage form	Daily serving size	Mass of serving size (g day^{-1})	Exposure to arsenic (ng day^{-1})					
				Total arsenic	Extractable arsenic	As^{3+}	As^{5+}	DMA	Unknown
P1	Tablet	3	4.0	1172 ± 66	947 ± 136	227 ± 35	–	605 ± 64	–
P2	Tablet	2	3.1	710 ± 82	580 ± 27	64 ± 11	–	378 ± 16	–
P3	Tablet	2	3.1	1078 ± 91	667 ± 33	112 ± 30	–	381 ± 97	–
P4	Capsule	3	2.7	1419 ± 90	984 ± 57	339 ± 54	–	312 ± 56	–
P5	Tablet	3	2.9	715 ± 58	631 ± 72	165 ± 17	62 ± 7	305 ± 22	–
P6	Capsule	2	2.7	535 ± 38	381 ± 11	74 ± 6	–	328 ± 20	–
P7	Tablet	4	6.8	1296 ± 160	790 ± 71	136 ± 31	–	527 ± 58	–
C1	Tablet	1[a]	3.1[a]	450 ± 76[a]	410 ± 38[a]	144 ± 30	–	271 ± 49	–
		0.5[b]	1.6[b]	225 ± 38[b]	205 ± 19[b]	72 ± 15	–	136 ± 24	–
C2	Tablet	2	3.1	1582 ± 108	1358 ± 91	109 ± 5	208 ± 26	383 ± 18	353 ± 79
C3	Liquid	2[c]	6.6	391 ± 29	356 ± 40	165 ± 14	–	109 ± 15	–

[a] Children of 4 years and older.
[b] Children younger than 4 years of age.
[c] Tea spoon.

see Table 6. The serving mass was derived from the recommended number of pills per day as written on the product labels. Pregnant women who consume any of the prenatal supplements (P1–P7) are exposed to 535–1419 ng total arsenic per day. Considering the individual arsenic species, supplements P1–P7 expose their consumers to 64–339 ng As^{3+}, 62 ng As^{5+} and 305–605 ng DMA daily. For a woman of 55 kg body weight, consumption of these supplements would lead to exposure of 10–26 ng total arsenic per kg body weight per day. The highest arsenic exposure was found to be from product P4 where fruits and vegetables are the main ingredients (see Table 2). The daily exposure of children who take any of the supplement products (C1–C3) is 225–1582 ng total arsenic. If the individual arsenic species are considered, the children are exposed to 72–165 ng As^{3+}, 208 ng As^{5+} and 109–383 ng DMA. Supplement C2, which has cherry and other natural substances as main ingredients, causes the highest exposure. For a four-year-old child of 18 kg body weight, consumption of supplements C1–C3 would cause exposure to 13–88 ng total arsenic per kg body weight per day. Comparison of these exposure values with the benchmark dose levels set by some regulatory bodies shows that the values are below the limits set by FAO and WHO (0.3–8 μg inorganic arsenic per kg of body weight per day) [15], as well as the US EPA [16] and the State of California [17] (10 μg arsenic per day).

4. Conclusions

The present study has demonstrated the determination of arsenic species in some widely consumed prenatal and children's dietary supplements using microwave-enhanced extraction and IC–ICP-MS analysis. It is shown that a solution of H_3PO_4 can be used to extract arsenic from these products without significantly affecting the stability of the species of the element. A mass balance comparison between total arsenic and the sum of extracted and non-extracted arsenic verified the validity of the analytical procedures. The application of the study is of great importance particularly to vulnerable population subgroups; pregnant women and children. The study found that all the analyzed dietary supplements contain arsenic mainly as the carcinogenic and neurotoxic As^{3+} and as DMA, which is a cancer promoter. Although these products expose their consumers to arsenic below the tolerance limits set by some regulatory bodies, the presence of highly toxic forms of the element in the supplements should alert manufacturers to control the quality of their products and provide safe, consistent and reliably high quality supplements to the public. This can be achieved by monitoring the raw material plant components and extracts, the additives, the manufacturing process, and the final products. Such activities necessitate the use of efficient analytical methods; the present study is of high relevance since methods for arsenic speciation analysis in such products are lacking. In addition, safety, quality information and advice should be publicly available to the producers, traders and consumers of supplementary products in relation to the quality of the materials and the risks associated with contamination by arsenic and other toxic and xenobiotic substances. Protocols and strategies must be developed to help the entire chain, from the farm and factory to the final product, reduce or eliminate toxins for the most critical and vulnerable

period: preconception, conception and early development years of infants.

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